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Rapid Report

Evidence that the F_2-isoprostane, 8-epi-prostaglandin $F_{2\alpha}$, is formed in vivo

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F_2-isoprostanes are prostaglandin (PG)F_2-like compounds that are produced in vivo as non-enzymatic products of free radical catalyzed peroxidation of arachidonic acid. One F_2-isoprostane whose formation should be favored is 8-epi-$PGF_{2\alpha}$. 8-Epi-$PGF_{2\alpha}$ has been shown to exert potent bioactivity but proof that it is formed in vivo is lacking. Evidence is now presented suggesting that 8-epi-$PGF_{2\alpha}$ is, in fact, formed in vivo by demonstrating that an endogenous F_2-isoprostane with a retention time on capillary GC identical with that of 8-epi-$PGF_{2\alpha}$ co-chromatographs through four high resolving HPLC purification procedures with authentic radiolabelled 8-epi-$PGF_{2\alpha}$.

Free radicals have been implicated in the pathogenesis of a wide variety of human disorders. One of the major targets of free radical injury are lipids which undergo peroxidation. Recently, we reported the discovery that a series of prostaglandin (PG)F_2-like compounds (F_2-isoprostanes) are produced in vivo as products of free radical catalyzed peroxidation of arachidonic acid [1]. Formation of these compounds occurs independently of the cyclooxygenase enzyme and proceeds through intermediates comprised of peroxyl radical isomers of arachidonic acid which undergo endocyclization to form endoperoxides. The endoperoxides are then reduced to yield F_2-isoprostanes. Four positional isomers of F_2-isoprostanes are formed, each of which can be theoretically comprised of 8 racemic diastereomers.

Based on the mechanism of formation of F_2-isoprostanes, one compound whose formation would be favored is 8-epi-$PGF_{2\alpha}$ [1]. There has been considerable interest in this molecule because several recent studies have shown that it exerts potent biological activity. For example, 8-epi-$PGF_{2\alpha}$ has been found to be a potent vasoconstrictor in both the lung and kidney and is one of the most potent renal vasoconstricting substances yet identified [1–4]. In addition, we recently reported the interesting findings that 8-epi-$PGF_{2\alpha}$ appears to cause vasoconstriction via an interaction with a unique receptor on vascular smooth muscle and inhibits thromboxane receptors on platelets [5,6]. In spite of the fact that considerable work has been carried out investigating the biological activity of 8-epi-$PGF_{2\alpha}$, definitive evidence that it is actually formed in vivo is lacking. Previous work has suggested that 8-epi-$PGF_{2\alpha}$ might be formed in vivo in that F_2-isoprostane containing extracts of biological fluids contain compounds with similar properties on thin layer chromatography and gas chromatography (GC) as authentic 8-epi-$PGF_{2\alpha}$ [1]. However, as many as 64 different F_2-isoprostanes can theoretically be formed and purification of F_2-isoprostanes by thin layer chromatography only provides an incompletely resolved mixture of these compounds.

For obvious reasons, information as to whether 8-epi-$PGF_{2\alpha}$ is actually produced in vivo is essential to evaluate the relevance of recent studies that have begun to investigate the biological activity of this compound and whether future studies exploring its biological actions will be of any importance. To obtain such evidence, we assessed whether an F_2-isoprostane was present in a biological extract that has a retention time on capillary GC identical to that of chemically synthesized 8-epi-$PGF_{2\alpha}$ and co-chromatographs with radiolabelled 8-epi-$PGF_{2\alpha}$ through four different high resolving high pressure liquid chromatography (HPLC) purification procedures.

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Abbreviations: PG, prostaglandin; GC, gas chromatography; HPLC, high pressure liquid chromatography; MS, mass spectrometry; PFB, pentaflurobenzyl ester.

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A mixture of F_2-isoprostanes was isolated from the liver of a Sprague-Dawley rat which had been treated with CCl_4 (2 ml/kg) to induce lipid peroxidation as described previously and outlined briefly below [7,8]. We recently reported the interesting finding that, unlike cyclooxygenase-derived prostaglandins, F_2-isoprostanes are initially formed in situ esterified to phospholipids and subsequently released in free form by a phospholipase(s) [9]. Therefore, crude extracts containing F_2-isoprostanes esterified to phospholipids were initially isolated by Folch extraction of 2 g of liver. The lipid extract was then subjected to hydrolysis with methanolic KOH. Free F_2-isoprostanes were extracted and partially purified using C18 and silica Sep-Pak cartridges (Waters Associates, Milford, MA) [10].

Quantification of F_2-isoprostanes in the partially purified liver extract and throughout subsequent HPLC purification procedures was performed by analyzing aliquots by selected ion monitoring (GC) negative ion chemical ionization/mass spectrometry (MS) employing $[^2H_4]PGF_{2\alpha}$ as an internal standard as described [10]. Compounds were analyzed as pentafluorbenzyl (PFB) ester, trimethylsilyl ether derivatives monitoring the M-PFB (M-181) ions m/z 569 for endogenous F_2-isoprostanes and m/z 573 for $[^2H_4]PGF_{2\alpha}$. Selected ion current chromatograms obtained from the analysis of the hydrolysate of the initial liver extract prior to HPLC purification is shown in Fig. 1. As is evident, several m/z 569 peaks are present representing a mixture of different F_2-isoprostanes. Synthetic 8-epi-$PGF_{2\alpha}$ (a generous gift of Dr. Gordon Bundy, Upjohn Co., Kalamazoo, MI) co-chromatographs with the starred (*) peak in the m/z 569 chromatogram. The intensity of this peak was used to quantify F_2-isoprostanes throughout the experiments described herein.

To the partially purified hydrolysate containing the mixture of F_2-isoprostanes (approximately 1350 ng) was added 0.3 μCi of $[^3H_1]$ 8-epi-$PGF_{2\alpha}$ (75 Ci/mmol) (custom synthesized by Dupont New England Nuclear, Boston, MA). The mixture was then subjected to four successive HPLC purification steps. To maximize purification and resolution of the different F_2-isoprostanes, HPLC procedures were developed that yielded relatively long retention volumes for 8-epi-$PGF_{2\alpha}$ (approximately 30 ml) and each solvent system was run isocratically. In addition, small fraction volumes (0.33–0.50 ml) were collected for analysis to provide several data points over the eluted radiolabelled 8-epi-$PGF_{2\alpha}$ peak. The radiolabelled 8-epi-$PGF_{2\alpha}$ contains only a single tritium atom at C-9. Thus it would not be expected that the tritiated 8-epi-$PGF_{2\alpha}$ and unlabelled 8-epi-$PGF_{2\alpha}$ would be discriminated and separated to a great degree by HPLC purification. Indeed, this was confirmed by chromatographing a mixture of radiolabelled and unlabelled 8-epi-$PGF_{2\alpha}$ isocratically on both normal and reversed phase HPLC and analyzing aliquots of fractions eluted both for radioactivity and for unlabelled 8-epi-$PGF_{2\alpha}$ by GC/MS. Both radiolabelled and unlabelled compounds co-chromatographed on HPLC with only a slight separation of up to 0.33 ml.

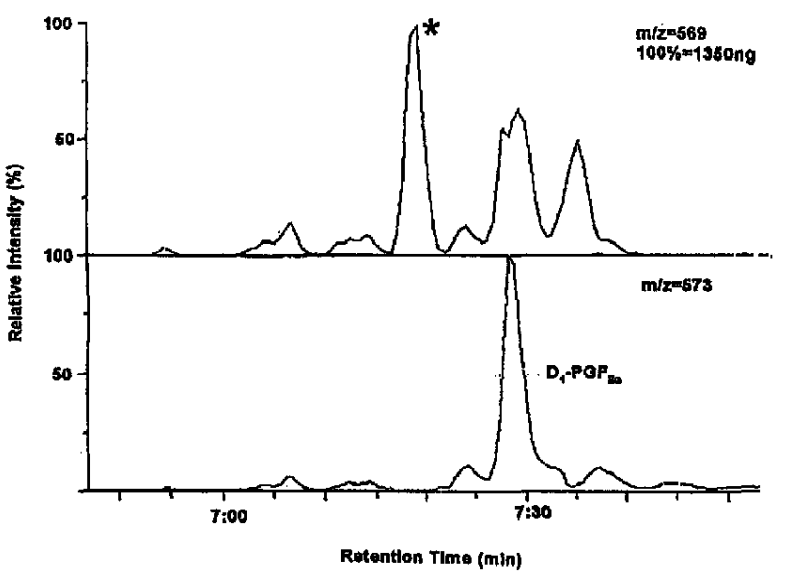

Fig. 1. Selected ion current chromatograms obtained from the analysis a crude hydrolyzed liver extract for F_2-isoprostanes. The m/z 569 chromatogram shows a series of peaks representing endogenous F_2-isoprostanes and the m/z 573 chromatogram represents the $[^2H_4]PGF_{2\alpha}$ internal standard. Quantification of the F_2-isoprostanes is based on the relative intensity of the starred (*) peak in the m/z 569 chromatogram to the internal standard. The starred (*) m/z 569 peak also has an identical GC retention time as synthetic 8-epi-$PGF_{2\alpha}$.

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The crude liver hydrolysate containing the mixture of F_2-isoprostanes was initially subjected to normal phase HPLC purification using a 25 cm × 4.6 mm Econsil SI column with 5 μm particles (Alltech Associates, Deerfield, IL). The solvent system was hexane/isopropyl alcohol/acetic acid (85 : 15 : 0.1, v/v/v) which was run isocratically at a flow rate of 1 ml/min. Aliquots of fractions eluted from the HPLC were then analyzed for F_2-isoprostanes by GC/MS and for radioactivity (Fig. 2A). Radiolabelled 8-epi-$PGF_{2\alpha}$ eluted in this system between 25–28 ml. Interestingly, compounds representing endogenous F_2-isoprostanes were present which had a retention time on GC the same as that of 8-epi-$PGF_{2\alpha}$ but which eluted with different retention volumes from that of 8-epi-$PGF_{2\alpha}$ on HPLC. Importantly, however, as shown in Fig. 2A, an endogenous F_2-isoprostane peak was also detected that co-eluted with the radiolabelled 8-epi-$PGF_{2\alpha}$ peak in this HPLC system.

Material eluted from the HPLC between 25.5–27.5 ml in Fig. 1A was then subjected to reversed phase HPLC using an Econosil C18 column (25 cm × 4.6 mm, 5 μm particles (Alltech Associates)). The mobile phase was acetonitrile/water/acetic acid (28 : 72 : 0.1, v/v/v) which was run isocratically at a flow rate of 1 ml/min. Aliquots of fractions collected were again analyzed for endogenous F_2-isoprostanes by GC/MS and for radioactivity (Fig. 2B). Radiolabelled 8-epi-$PGF_{2\alpha}$ eluted from the HPLC with a retention volume of 29–30.5 ml. Analysis of aliquots of the eluted fractions by GC/MS showed that almost all the unlabelled F_2-isoprostane material detected in the chromatogram co-eluted on HPLC with radiolabelled 8-epi-$PGF_{2\alpha}$ with the exception of a small additional amount of material which eluted at the trailing end (note shoulder) of the radiolabelled 8-epi-$PGF_{2\alpha}$ peak.

It is well recognized that altering the polarity of a compound by derivatization and rechromatographing

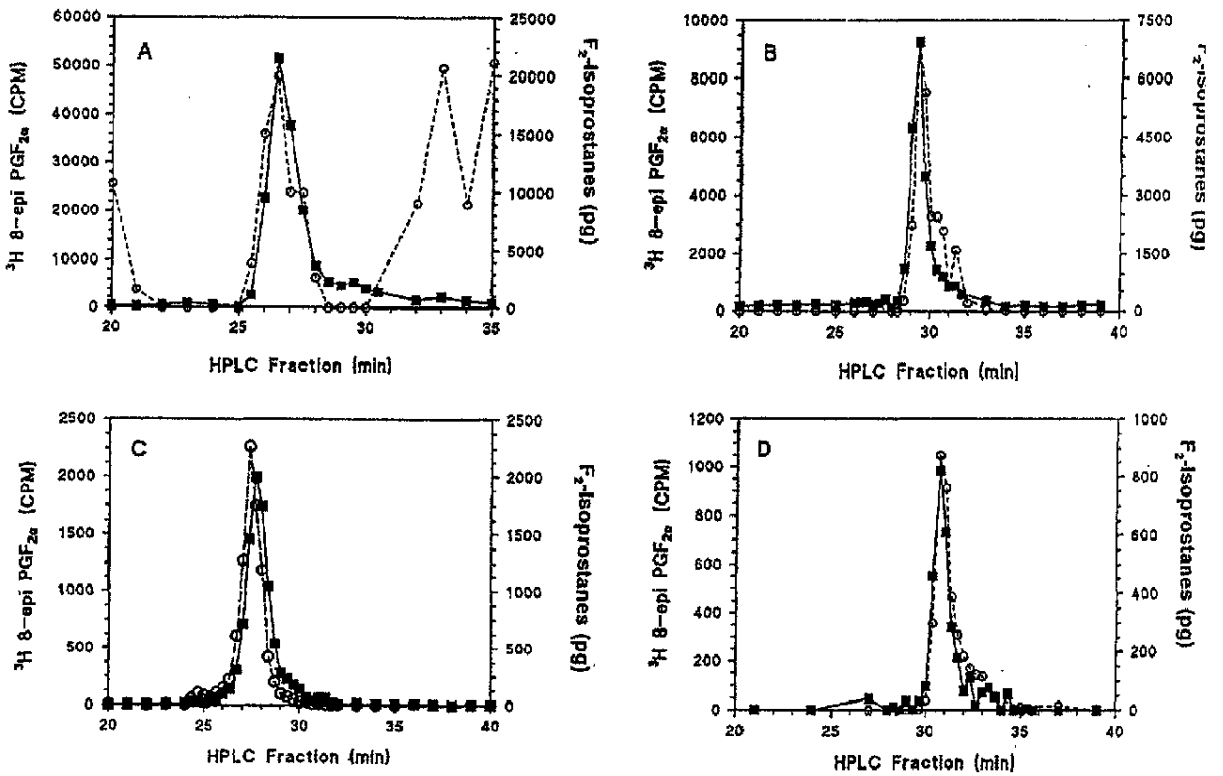

Fig. 2. HPLC purification of the mixture of F_2-isoprostanes shown in Fig. 1. Details regarding solvent systems used are described in the text. Tritiated 8-epi-$PGF_{2\alpha}$ was added to the mixture at the beginning and aliquots of fractions eluted were assayed for radioactivity (closed squares, solid lines). Aliquots were also assayed and quantified by GC/MS for the presence of an F_2-isoprostane peak with the same GC retention time as that for authentic 8-epi-$PGF_{2\alpha}$ (open circles, dotted lines). All HPLC purification procedures were carried out isocratically. (A) Normal phase HPLC as free acids of the initial mixture of F_2-isoprostanes shown in Fig. 1. (B) Reversed phase HPLC as free acids of the material eluted at the retention volume between 25 and 27.5 ml in Fig. 2A. (C) Normal phase HPLC as PFB esters of the material eluted at the retention volume between 29 and 30 ml in Fig. 2B. (D) Reversed phase HPLC as PFB esters of the material eluted at the retention volume between 27 and 28.33 ml in Fig. 2C.

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the compound can provide a powerful approach for purification and separation of biomolecules. Therefore, the material eluted from the HPLC between 29–30 ml in Fig. 2B was converted to a pentafluorbenzyl (PFB) ester and rechromatographed on normal phase HPLC. Pooling only the material eluted between 29–30 ml eliminated most of the material eluting as a shoulder on the trailing edge of the radiolabelled 8-epi-$PGF_{2\alpha}$ peak in Fig. 2B. A solvent system of hexane/isopropyl alcohol (88:12, v/v) was used which was run isocratically at a flow rate of 1 ml/min. Fractions were again collected and analyzed for radioactivity and by GC/MS (Fig. 2C). The radiolabelled PFB ester of 8-epi-$PGF_{2\alpha}$ eluted between 27–29 ml. As before, unlabelled material was present that eluted within the expected limit of separation of labelled and unlabelled 8-epi-$PGF_{2\alpha}$ on HPLC.

Material eluted between 27–28.33 ml in the HPLC step shown in Fig. 2C was then pooled. This material was then subjected to further purification by reversed phase HPLC using a solvent system of acetonitrile:water (50:50, v/v) which was run isocratically at a flow rate of 1 ml/min. The results of the analyses for radioactivity and endogenous F_2-isoprostanes in the eluted fractions are shown in Fig. 2D. Only a single unlabelled PFB ester F_2-isoprostane peak eluted from the HPLC column which co-eluted with the PFB ester of radiolabelled 8-epi-$PGF_{2\alpha}$. In addition, when analyzed by GC/MS, only a single F_2-isoprostane peak was present (Fig. 3). Further, when this material was mixed with an approximate equivalent amount of derivatized synthetic 8-epi-$PGF_{2\alpha}$ and analyzed by GC/MS, the two compounds perfectly co-chromatographed on capillary GC without any suggestion of a shoulder on the GC peak (not shown).

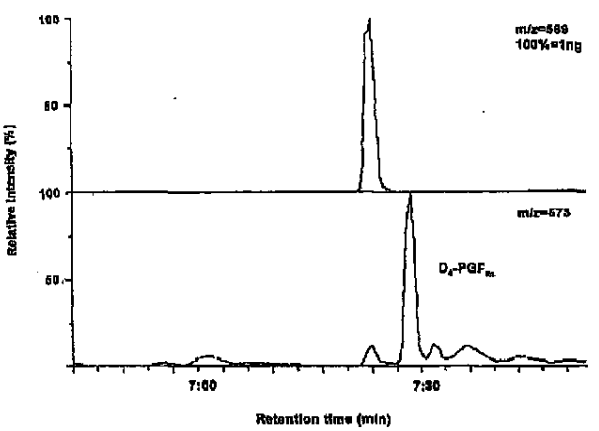

Fig. 3. Selected ion current chromatograms obtained from GC/MS analysis of the material eluted at a retention volume between 30 and 32 ml in Fig. 2D. Only a single m/z 569 peak representing an endogenous F_2-isoprostane, presumably 8-epi-$PGF_{2\alpha}$, remains after the four HPLC purification procedures shown in Fig. 2. The peak in the lower m/z 573 chromatogram represents the deuterated $PGF_{2\alpha}$ internal standard. The peak in the m/z 569 chromatogram was found to perfectly co-chromatograph when analyzed as a mixture with an approximate equivalent amount of synthetic 8-epi-$PGF_{2\alpha}$.

The one analytical approach that would unequivocally prove that the F_2-isoprostane that co-eluted with radiolabelled 8-epi-$PGF_{2\alpha}$ in the final HPLC purification step (Fig. 2D) is, in fact, 8-epi-$PGF_{2\alpha}$ would be NMR spectroscopy. However, it would not be possible to obtain a sufficient quantity of this compound from a biological source for analysis by NMR. Given these limitations, however, the results of the studies described herein would seem to provide evidence as best that can be obtained that 8-epi-$PGF_{2\alpha}$ is one of the F_2-isoprostanes that is produced in vivo. The abundance of 8-epi-$PGF_{2\alpha}$ formed relative to that of the other F_2-isoprostanes can also be roughly estimated from the results obtained. At the beginning, a known amount of radiolabelled 8-epi-$PGF_{2\alpha}$ was added to the crude liver hydrolysate containing a mixture of F_2-isoprostanes and the intensity of the peak in this mixture with a GC retention time the same as that of 8-epi-$PGF_{2\alpha}$ (starred peak in Fig. 1) was quantified. As shown in Fig. 2, some of the material in the starred peak in Fig. 1 was subsequently separated during the HPLC purification steps. As a result, after the fourth HPLC, the ratio of the amount of material present when quantified by GC/MS to the amount of radioactivity recovered had been reduced to an extent that indicated that 8-epi-$PGF_{2\alpha}$ represented approximately 1/4 to 1/3 of the total amount of the original material present in the starred peak in Fig. 1. Of note, however, is that this peak is the most prominent peak present in the mixture of F_2-isoprostanes which suggests that 8-epi-$PGF_{2\alpha}$ may be one of the more abundant F_2-isoprostanes formed.

In summary, we have provided evidence that 8-epi-$PGF_{2\alpha}$ is one of the F_2-isoprostanes that are produced in vivo. This finding is of particular importance, since recent studies have shown that 8-epi-$PGF_{2\alpha}$ exerts potent biological activity suggesting that 8-epi-$PGF_{2\alpha}$ could participate as a mediator in the pathophysiology of oxidant injury. In light of these findings, future studies aimed at exploring further the spectrum of the biological activity of this isoprostane and the mechanisms by which it exerts these actions should also, therefore, be of relevance.

Acknowledgements

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References

1 Morrow, J.D., Hill, K.E., Burk, R.F., Nammour, T.M., Badr, K.F. and Roberts, L.J. II (1990) Proc. Natl. Acad. Sci. USA 87, 9383–9387.
2 Takahashi, K., Nammour, T.M., Ebert, J., Morrow, J.D., Roberts, L.J. II and Badr, K.F. (1992) J. Clin. Invest. 90, 136–141.
3 Banerjee, M., Kang, K.H., Morrow, J.D., Roberts, L.J. II and Newman, J.H. (1992) Am. J. Physiol. 263, H660–H663.
4 Kang, H.K., Morrow, J.D., Roberts, L.J. II, Newman, J.H. and Banerjee, M. (1993) J. Appl. Physiol. 74, 460–465.
5 Fukunaga, M., Makita, N., Roberts, L.J. II, Morrow, J.D., Takahashi, K. and Badr, K. (1993) Am. J. Physiol. 264, C1619-C1624.
6 Morrow, J.D., Minton, T.A. and Roberts, L.J. II (1992) Prostaglandins 44, 155–163.
7 Morrow, J.D., Awad, J.A., Kato, T., Takahashi, K., Badr, K.F., Roberts, L.J. II and Burk, R.F. (1992) J. Clin. Invest. 90, 2502–2507.
8 Radin, N.S. (1969) Methods Enzymol. 14, 245–248.
9 Morrow, J.D., Awad, J.A., Boss, H.J., Blair, I.A. and Roberts, L.J. II (1992) Proc. Natl. Acad. Sci. USA 89, 10721–10725.
10 Morrow, J.D., Harris, T.M. and Roberts, L.J. II (1990) Anal. Biochem. 184, 1–10.

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